Table of Contents

8.0 Other Scientific Reports

8.1 Summaries of Available Data from Studies using the BG1Luc ER TA

This section reviews published studies that used BG1Luc4E2-based ER TA test methods to evaluate estrogen agonist or antagonist activity of a number of substances. Results for many of the substances described in studies presented by Gordon et al. 2003 and 2004 (see **Sections 8.1.3** and **8.1.4** below) were also provided in the XDS submission (**Annex A**). Additionally, a separate study that compared the relative utility of qualitative and quantitative methods for determining BG-1 cell viability during the assay is described.

8.1.1 Rogers and Denison (2000)

Rogers and Denison (2000) describes the original development, optimization, and characterization of the BG-1 cell line, a stably transfected, recombinant human ovarian cancer cell line. BG-1 cells were transfected using the pGudLuc7.0 plasmid containing a segment of the pGudLuc1.0 vector with hormone response elements, and the mouse mammary tumor viral promoter, but lacking glucocorticoid-responsive elements. The parent vector, pGudLuc7.0, was shown to be unresponsive to estrogen in BG-1 cells. After demonstration of estrogen responsive luciferase activity in transiently-transfected cells, a stably transfected, estrogen-responsive BG-1 clone was isolated and designated BG1Luc4E2. BG1Luc4E2 displayed constitutive activation of the luciferase gene under normal culture conditions, but this activity was greatly reduced when cells were grown in estrogen-stripped media (EFM). The estrogen responsive induction of luciferase seen in BG1Luc4E2 cells that are grown in EFM is time and dose-dependent. While maximal induction following exposure to 0.1nM estradiol was seen at 20 hours, the minimum detection limit was between 0.1 – 1pM estradiol. Cross reactivity of BG1Luc4E2 cells with six other steroid hormones was also evaluated. Progesterone, testosterone, all-trans retinoic acid, and thyroid hormone did not induce luciferase activity, but dihydrotestosterone and dexamethasone produced slight induction (based on three independent experiments where substances were considered positive for ER TA agonist activity when induction of luciferase was significantly different from control, at $p<0.05$ as determined by a *t* test).

8.1.2 Jefferson et al. (2002)

This paper (Jefferson et al. [2002]) describes a study that evaluated the ER TA activities of several phytoestrogens (genistein, daidzein, coumestrol, zearalanol, zearalenone, naringenin, taxifolin, and biochanin A) using a BG1Luc4E2-based test method. All substances except taxifolin tested positive for ER TA activity with EC_{50} values (μg/mL) ranging from 3.9×10^{-5} (zearalanol) to 1.2×10^{0} μg/mL

(naringenin) as compared to 17β–estradiol (2.3×10^{-6} μg/mL) or diethylstilbestrol (4.9×10^{-6} μg/mL). The specific criteria used to determine negative ER TA response and number of tests per substance was not provided. ER TA results were compared to uterotrophic bioassay results for the substances and showed agreement for all substances except daidzein and naringenin, which were weakly positive for ER TA activity (3.9×10^{-5} and 3.9×10^{-5} μg/mL respectively) but negative when tested in the uterotrophic bioassay.

8.1.3 Gordon et al. (2003)

The 2003 International Dioxin Symposium (Boston, MA) presentation of Gordon et al. (2003) describes studies that evaluated the ER TA activities of 78 substances using a BG1Luc4E2-based test method. Of these substances, 29 had been previously tested in other ER TA assays (which were not identified in the presented paper). The remaining 49 substances, which were classified by the presenter as environmental contaminants, had not been previously tested in ER TA assays. All substances were tested independently at least three times and ER TA activity was based on whether induction of luciferase was less or greater than three times the standard deviation (SD) of the mean vehicle control value. Using these criteria, 61 substances were positive and 17 were negative for ER TA activity (note: a complete listing of results for individual substances was not provided, graphical presentation of concentration response curves for 12 positive and 3 negative substances was provided as representative examples). Results also indicated that the 29 substances previously tested were in agreement with the BG1Luc ER TA test method results, with the exception of progesterone, which was negative in the BG1Luc ER TA, but positive in other ER TA test methods.

8.1.4 Gordon et al. (2004)

The 2004 International Dioxin Symposium (Berlin, Germany) presentation of Gordon et al. (2004) describes studies that evaluated the ER TA activity of 13 commonly used organochlorine pesticides using a BG1Luc4E2-based test method. Each substance was tested independently at least three times and ER TA activity was based on whether induction of luciferase was less or greater than three times the SD of the mean vehicle control value. Based on these criteria, 11 substances were positive and 2 were negative for ER TA activity. EC_{50} values (M) for those testing positive for ER TA activity ranged from 1.3×10^{-6} (a-chlordane) to 1.2×10^{-5} (2,4,5-trichlorophenolxyacetic acid) as compared to 17β-estradiol (1.6×10^{-11}).

8.1.5 Gordon et al. (2005)

The 2005 International Dioxin Symposium (Toronto, Canada) presentation of Gordon et al. (2005) describes studies that evaluated the ER TA agonist activities of 10 commercially available sunscreens and 8 substances commonly used as “non-active” sunscreen components (substances that are not used to

protect against UV damage but rather as emulsifiers, emollients, lubricants, etc.) using a BG1Luc4E2 test method. The sunscreens and non-active sunscreen component substances were dissolved in methanol, serially diluted and evaluated for ER TA agonist activity. Each substance was tested independently at least three times and ER TA agonist activity was based on whether induction of luciferase was less or greater than three times the SD of the mean vehicle control value. This value was then translated into a 17β–estradiol equivalent of 10 ng/g to control for differences in extraction recovery for individual substances (i.e., substances with 17β–estradiol equivalents greater than 10 ng/g are considered positive for ER TA agonist activity). Nine of the 10 sunscreens tested positive for ER TA agonist activity but only 1 of the 8 non-active sunscreen component substances tested positive. The sunscreens testing positive for ER TA activity had a range of approximately 200 to 950 ng/g 17β–estradiol equivalents. The one non-active sunscreen component substance testing positive for ER TA activity (a substance used for water resistance) had a 17β–estradiol equivalent of 130 ng/g.

8.1.6 Clark et al. (2007)

The Clark et al. (2007) poster presentation from the 47th Annual Meeting of the Society of Toxicology Meeting (Charlotte, NC) describes a study that was conducted using the BG1Luc ER TA to determine if a qualitative method of assessing cell viability based on a visual observation was comparable to Promega Corporation's CellTiter-Glo® quantitative cell viability assay, which measures cell viability based on the generation of luminescence signal proportional to the amount of ATP in viable cells. The qualitative visual observation method is based on an assessment of cell density and morphology and the criteria for assessing and scoring cell viability is provided in **Table 1**.

Table 8-1 Visual Observation Scoring Table

Viability Score	Brief Description[1]
1	Normal Cell Morphology and Cell Density
2	Altered Cell Morphology and/or Small Gaps between Cells
3	Altered Cell Morphology and/or Large Gaps between Cells
4	Few (or no) Visible Cells

Comparison of the two cell viability assessment methods demonstrated that a score of one in the visual observation method corresponded to greater than 80% viability in the CellTiter-Glo® assay, and visual observation scores of 2, 3, and 4 corresponded to 80 – 60%, 60 – 40%, and less than 40%, respectively in the CellTiter-Glo® assay. An assessment of cell viability is critical in determining whether reduction of ER TA activity is either ER mediated or the result of cytotoxicity and the study showed that the visual observation method and CellTiter-Glo® assay are comparable for this assessment. Importantly, these

results demonstrated that the simpler and more economical visual observation method can be used as effectively as the more complex and costly CellTiter-Glo®, which requires testing on separate parallel plates.

Clark GC, Gordon JD, Denison MS, Chu AC, Chu MD, Matherly C, et al. 2007. Comparison of Visual Inspection to CellTier-Glo® in Evaluating Cytotoxicity in theLUMI-CELL® ER Bioassay. SOT 2007: 46th Annual Meeting & ToxExpo™, Charlotte, North Carolina, March 25-29, 2007.

Gordon JD, Chu AC, Chu MD, Denison MS, Clark GC. 2004. Detection of Estrogen Receptor Endocrine Disruptor Potency of Commonly Used Organochlorine Pesticides Using The LUMI-CELL ER Bioassay. Dioxin 2004: 24th International Symposium on Halogenated Environmental Organic Pollutants and Persistent Organic Pollutants, Berlin, Germany September 6-10, 2004 Organohalogen Compounds 66: 169-174.

Gordon JD, Chu AC, Chu MD, Matherly C, Denison MS, Grune G, et al. 2005. Estrogenic Potency of Many Popular Sunscreens and It's "Non-Active" Components Detected using the LUMI-CELL™ ER Bioassay. Dioxin 2005: 25th International Symposium on Halogenated Environmental Organic Pollutants and Persistent Organic Pollutants, Toronto, Canada August 21-26, 2005 Organohalogen Compounds 67: 2685-2689.

Gordon JD, Chu AC, Chu MD, Taylor CL, Denison MS, Clark GC. 2003. Validation of the LUMi-CELL™ ER Recombinant Bioassay for Rapid Evaluation of Chemicals for Potential Estrogenic Activity. Dioxin 2003: 23rd International Symposium on Halogenated Environmental Organic Pollutants and Persistent Organic Pollutants, Boston, Massachusetts August 24-29, 2003 Organohalogen Compounds 65: 78-81.

Jefferson WN, Padilla-Banks E, Clark G, Newbold RR. 2002. Assessing estrogenic activity of phytochemicals using transcriptional activation and immature mouse uterotrophic responses. Journal of Chromatography B 777(1-2): 179-189.

Rogers JM, Denison MS. 2000. Recombinant cell bioassays for endocrine disruptors: Development of a stably transfected human ovarian cell line for the detection of estrogenic and anti-estrogenic chemicals. In Vitr Mol Toxicol 13(1): 67-82.